Contains Nonbinding Recommendations

Draft Guidance on Cholestyramine

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Active ingredient: Cholestyramine

Form/Route: Powder/Oral

Recommended studies: 2 In Vitro Studies

1. Type of study: In-vitro equilibrium binding study
   Design: With and without acid pre-treatment at pH 6.8
   Strength: Equivalent to 4 gm resin/packet or equivalent to 4 gm resin/scoopful
   Subjects: Not applicable
   Additional Comments: The equilibrium binding study is considered the pivotal bioequivalence study. This study should be conducted by incubating the Test and Reference products with at least eight different concentrations of total bile salts, with and without acid pretreatment. Each bile salt-containing incubation medium should contain glycocholic acid (GCA), glycochenodeoxycholic acid (GCDA) and taurodeoxycholic acid (TDCA). Total bile salt concentrations should be spaced along the spectrum until the maximum binding is clearly established. In addition, data should be provided demonstrating that the length of time selected for incubation with the total bile salt-containing medium yields maximum binding. See below for details on the study design.

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2. Type of study: In-vitro kinetic binding study
   Design: Without acid pre-treatment
   Strength: Equivalent to 4 gm resin/packet or equivalent to 4 gm resin/scoopful
   Subjects: Not applicable
   Additional Comments: The kinetic binding study should be used to support the pivotal equilibrium binding study. This study should be conducted by incubating the Test and Reference products for at least eight different lengths of time, with two different constant total bile salt concentrations, without acid pre-treatment. The total bile salt concentrations used should be the lowest and highest used in the equilibrium binding study without acid pre-treatment. Times should be selected along the spectrum until the maximum binding is clearly established.

   See below for details on the study design.

Analytes to measure: Unbound bile salts in filtrate (to calculate bile salts bound to resin).

For the *in vitro* equilibrium binding study, the Langmuir binding constants k_1 and k_2 should be determined based on total bile salt binding (GC+GCDC+TDC). The test/reference ratio should be calculated for k_1. The 90% confidence interval should be calculated for k_2 with the acceptance criteria of 80% to 120%.

For the *in vitro* kinetic binding study, the test/reference bound bile acid salt ratios at the various times should be compared but not subjected to the 90% confidence interval criteria.

Bioequivalence based on (90% CI): The Langmuir binding constant k_2 from the equilibrium binding study.

Waiver requests of in vivo and in-vitro testing: Not applicable.

Please note that the Orange Book designates two reference products each in 2 different presentations (packet and scoopfuls): Cholestyramine and Cholestyramine Light (sugar free). Two separate applications and separate studies must be submitted comparing to the appropriate reference product (you may choose either presentation). Please refer to the Guidance for Industry, *Variations in Drug Products that May Be Included in a Single ANDA* located at:
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064995.htm

Dissolution test method and sampling times: Not Applicable

Additional information regarding the In-Vitro Binding Study Protocols:

I. Protocol for Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF Without Acid Pre-treatment

Objective:

To compare the affinity and capacity binding constants of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulation under identical experimental conditions.

Materials:

1. Simulated intestinal fluid (SIF): 0.05 M potassium phosphate buffer solution without enzyme, pH 6.8, as specified in the USP.

2. Stock solution of bile acid salts in SIF: Prepare in SIF a 40 mM solution containing the sodium salts of the following bile acids in the molar proportion 3:3:1: glycocholic acid (GCA), 17.14 mM, glycochenodeoxycholic acid (GCDA), 17.14 mM, and taurodeoxycholic acid (TDCA), 5.72 mM.

3. Cholestyramine powder: generic formulation and reference drug product (Questran® or Questran Light®).

Procedures:

1. Incubation Mixtures for Test and Reference Drug Products.

   Set up eight incubation flasks of the test product and eight of the reference product, each containing the equivalent of 10 mg resin. Add 2 ml of SIF and soak at room temperature overnight. The following day, add to each container the requisite volumes of SIF and 40 mM bile acid salts solution in SIF to make the final volume of the solvent mixture 10 ml with the target concentrations of bile acid salts covering the ranges of 0.1 - 30 mM (Table 1).

TABLE 1: COMPOSITION OF INCUBATION MIXTURES AND CONCENTRATIONS OF INDIVIDUAL BILE ACID SALTS AT VARIOUS TARGET CONCENTRATIONS IN EQUILIBRIUM STUDY (ML STOCK SOLUTION + ML SIF + 2 ML SIF = 10 ML)

TARGET (mM)	STOCK (mL)	SIF (mL)	GCA (mM)	GCDCA (mM)	TDCA (mM)
0.1	0.025	7.975	0.0428	0.0428	0.0143
0.3	0.075	7.925	0.1286	0.01286	0.0429
1.0	0.250	7.750	0.4285	0.4285	0.1428
3.0	0.750	7.250	1.2855	1.2855	0.4284
7.0	1.750	6.250	2.9995	2.9995	0.9996
10.0	2.500	5.500	4.2850	4.2850	1.4280
20.0	5.000	3.000	8.5700	8.5700	2.8560
30.0	7.500	0.500	12.855	12.855	4.2840

2. Blank Incubation Mixture

   Prepare four blank incubation flasks each containing the drug product equivalent to 10 mg resin in 2 ml of SIF and soak at room temperature overnight. The next day add 8 ml of SIF to each blank. Two blanks will be used for the test and two blanks will be used for the reference product.

3. Standard Solutions of Bile Acid Salts

Dilute the requisite volumes of 40 mM stock solution of bile acid salts with SIF to yield 10 ml standard solutions of the following eight concentrations: 0.1, 0.3, 1, 3, 7, 10, 20, and 30 mM.

Incubation flasks for one set of experiments will thus include: 1) eight of the test product; 2) eight of the reference product; 3) four blanks; and 4) eight standards.

Incubate all 28 flasks at 37 C for 24 hours. Filter to collect the filtrate and assay the filtrate to determine concentrations of the bile acid salts. After incubation, the 0.1 mM standard solution filtrate is diluted with SIF to obtain the ninth standard with a concentration 0.05 mM.

The experiment should be repeated twelve times under the conditions described above to obtain twelve sets of data.

Data Treatment and Analysis

The amount of bile acid salt bound to cholestyramine resin is obtained from the difference between the initial concentration of bile acid salt introduced into the system and the concentration present in the filtrate at the end of the study. The monomolecular adsorption of adsorbate (bile acid salt) molecules from solution, at constant temperature, on to an adsorbent (cholestyramine resin) is described by the following Langmuir-type equation (5), Equation 1:

$$\frac{x}{m} = \frac{k_1 k_2 C_{eq}}{1 + k_1 C_{eq}}$$

Upon rearranging, Equation 2 is obtained:

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{C_{eq}}{k_2}$$

where:

Ceq = concentration of the adsorbate (bile acid salt) remaining in the solution at equilibrium;

x = the amount of adsorbate bound to the adsorbent (cholestyramine resin); and

m = the amount of adsorbent used. *Recommended*

The constant, **k1**, is defined as the adsorption coefficient or affinity constant and is related to the magnitude of the forces involved in the binding process.

The Langmuir-capacity constant, $\mathbf{k_2}$, indicates the apparent maximum amount of adsorbate that can be adsorbed per unit weight of adsorbent.

From the concentration of bile acid salt in the solution at equilibrium, the amount of bile acid salt, expressed in micromoles and as a percentage, bound to 10 mg of cholestyramine resin may be calculated. From this the amount of bile acid salt bound per mg of resin, the relationship **x/m** is calculated. A plot of **Ceq/(x/m) versus Ceq** on rectilinear coordinates should yield a straight line. Application of regression analysis will yield a slope (a) and intercept (b) of the line. The affinity constant, **k 1**, and capacity constant, **k 2**, are calculated from the slope and intercept as follows:

$$k_1 = a/b$$

$$k_2 = 1/a$$

Statistical packages with nonlinear regression programs are available that yield $\mathbf{k_1}$ and $\mathbf{k_2}$ values directly.

Parameters To Be Reported:

Twelve observations with **mean + SD** for the following parameters should be obtained and reported for both the test and reference products:

1. Percent binding of bile acid salt to 10 mg of resin at each concentration (tabular and graphical forms);

2. Micromoles of bile acid salts bound to 10 mg of resin at each concentration (in tabular and graphical forms);

3. Affinity constant, $\mathbf{k_1}$;

4. Capacity constant, $\mathbf{k_2}$;

5. Coefficient of determination, **r2**, when linear regression is used to determine $\mathbf{k_1}$ and $\mathbf{k_2}$.

II. Protocol for Equilibrium Study of Binding of Bile Acid Salts to Resin in SIF With

Acid Pre-treatment

Objective:

To compare the affinity and capacity constants of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulation after acid pre-treatment of both the products.

Materials:

1. 0.1 N hydrochloric acid

2. Other materials as in Section I

Procedures:

Soak the test and reference drug products equivalent to 10 mg of resin in 10 ml 0.1N hydrochloric acid at 37 C for 1 hour. Centrifuge and aspirate the supernate. Wash the drug product with SIF until pH 6.8 is attained. Soak the acid pre-treated resin product in 2 ml SIF at room temperature overnight. Conduct the remainder of the experiment as described in Section I.

III. Protocol for Study of Kinetics of Binding of Bile Acid Salts in 0.3mM Aqueous Solution in the Presence of Added Sodium Chloride (0.1 M)

Objective:

To compare the kinetics of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulations (Questran® and Questran Light®) under identical experimental conditions.

Materials:

1. Stock solution of sodium salts of bile acids: Prepare a 40 mM solution containing sodium salts of the following bile acids in the molar proportion 3:3:1 in water: glycocholic acid (GCA), 17.14 mM, glycochenodeoxycholic acid (GCDA), 17.14 mM, and taurodeoxycholic acid (TDCA), 5.72 mM.

2. 0.1 M sodium chloride in water.

3. 1.0 M solution of sodium chloride in water.

4. Cholestyramine powder: generic formulation and reference drug formulations (Questran® or Questran Light®).

Procedure:

1. Incubation Mixtures for the Test and Reference Drug Products

Soak the drug product equivalent to 10 mg resin in 2 ml of 0.1M sodium chloride solution at room temperature overnight. To this add quickly 0.075 ml of 40 mM bile acid salts solution in water, 0.8 ml of 1M sodium chloride stock solution, and 7.125 ml water to obtain a final volume of 10 ml fluid with a bile acid salts concentration of 0.3 mM. Prepare eight replicates of the incubation mixture as described above for the generic product and eight replicates for the reference drug products
(Table 2).

TABLE 2: COMPOSITION OF INCUBATION MIXTURES AND CONCENTRATIONS OF INDIVIDUAL BILE ACID SALTS AT TARGET CONCENTRATIONS OF 0.3 AND 3.0 mM IN KINETIC STUDY (ML STOCK SOLUTION + ML WATER + 0.8 ML 1M NACL + 2 ML 0.1M NACL = 10 ML)

TARGET (mM)	STOCK (mL)	WATER (mL)	1M NaCl (mL)	GCA (mM)	GCDCA (mM)	TDCA (mM)
0.3	0.075	7.125	0.80	0.1285	0.1285	0.043
3.0	0.750	6.450	0.80	1.286	1.286	0.428

2. Blank Incubation Mixtures

Soak the drug product equivalent to 10 mg of resin in 2 ml of 0.1 M solution of sodium chloride at room temperature overnight. Add 8 ml of 0.1 M sodium chloride solution, incubate for 24 hours at 37 C, filter, and collect the filtrate. At least two such blanks should be prepared for each drug product.

3. Standard Solutions of Bile Acid Salts

Prepare two standard solutions of bile acid salts of concentrations 0.1 and 0.3 mM in water by adding, to requisite volumes of 40 mM stock bile acid salts in water, 1 ml of 1.0 M stock sodium chloride solution and water to yield a final volume of 10 ml. Incubate the two standards at 37 C for 24 hours, filter, and collect the filtrate. Additional standards of concentrations 0.05 and 0.075 are obtained from the filtrate of 0.1 mM solution by dilution with 0.1 M sodium chloride solution. Additional standards of concentrations 0.15 and 0.21

mM are obtained from the filtrate of 0.3 mM solution by dilution with 0.1 M sodium chloride solution.

In one set of experiments, there will be eight incubation mixtures with the test product and eight with **each** of the reference products; six blank incubation mixtures; and two standard solutions of bile acid salts. Each of the incubation mixtures containing the test or reference drug product is incubated at 37 C for its designated time of incubation (0.25, 0.50, 1, 2, 4, 8, 16, or 24 hours), filtered, and the filtrate collected to determine the concentrations of the bile acid salts.

Data Treatment and Analysis:

The amount of bile acids salts bound to the resin is calculated from the initial concentrations of bile acid salts introduced into the system and the concentrations of bile acid salts present in the filtrate at the designated time points. From these values, bile acid salt bound to 10 mg of resin, expressed as percent and micromoles, at each time point are calculated.

The experiment should be repeated twelve times under the conditions described above to obtain twelve sets of data.

Parameters To Be Reported:

Twelve individual observations with **mean + SD** for the following parameters should be reported for both the test and reference products:

1. Percent binding of bile acid salt to 10 mg of resin at each time point in tabular and graphical forms; and

2. Micromoles of bile acid salt bound to 10 mg of resin at each time point in tabular and graphical forms.

IV. Protocol for the Study of Kinetics of Binding of Bile Acid Salts in 3 mM Aqueous Bile Acid Salts Solution in the Presence of Added Sodium Chloride (0.1 M).

Objective:

To compare the kinetics of binding of bile acid salts to cholestyramine resin in a generic formulation with that in the reference formulations (Questran® and Questran Light®) under identical experimental conditions.

Materials:

1. Stock solution of sodium salts of bile acids in water: prepare as described in Section III.

2. A solution of 0.1 M sodium chloride in water.

3. A solution of 1.0 M sodium chloride in water.

4. Cholestyramine powder: generic formulation and reference drug products (Questran® and Questran Light®)

Procedures:

1. Incubation Mixtures for the Test and Reference Drug Products

   Soak the drug product equivalent to 10 mg resin in 2 ml of 0.1 M sodium chloride solution at room temperature overnight. To this add quickly 0.75 ml of 40 mM bile acid salts solution in water, 0.8 ml of 1 M of sodium chloride stock solution, and 6.45 ml water to obtain a final volume of 10 ml and bile acid salts concentration of 3.0 mM. Prepare eight replicates of the incubation mixture each for the generic product and the two reference products.

2. Blank Incubation Mixtures

   Prepare as described in section III above.

3. Standard Solutions of Bile Acid Salts

   Prepare two standard solutions of bile acid salts of concentrations 0.1 and 3.0 mM in water by adding, to requisite volumes of 40 mM stock solution of bile acid salts in water, 1 ml of 1.0 M stock solution of sodium chloride and water to make the final volume 10 ml. Incubate the two standards at 37 C for 24 hours. Additional standards of 0.05 and 0.075 mM are obtained from the filtrate of 0.1 mM solution. Additional standards of 0.3 and 1.0 mM are obtained from the filtrate of 3.0 mM solution by dilution with 0.1 M sodium chloride solution.

   In one set of experiments, there will be eight incubation mixtures for the test product, eight with each of the reference products, six blank incubation mixtures, and two standard solutions of bile acid salts. Each of the incubation mixtures containing the test or the reference drug product is incubated at 37°C for its designated time of incubation (0.25, 0.50, 1, 2, 4, 8, 16, or 24 hours), filtered, and the filtrate collected to determine the concentrations of bile acid salts.

   The experiment should be repeated twelve times under the conditions described above to obtain twelve sets of data.

Data Treatment and Analysis:

As in Section III above.

G. Facilities

The analytical facility used for the study should be identified. The names, titles, and

curriculum vitae of the scientific/analytical directors should be included in the study report.

REFERENCES

1. Johns WM , Bates TR. Quantification of the Binding Tendencies of Cholestyramine I: Effect of Structure and Added Electrolytes on the Binding of Unconjugated and Conjugated Bile-Salt Anions. J Pharm Sci 1969; 58:179-183.

2. Graham DY, Sackman JW, Giesing DH, Rusner DJ. *In vitro* adsorption of bile salts and aspirin to sucralfate. Digestive Diseases and Sciences 1984; 29:402-6.

3. Kos R, White JL, Hem SL, Borin MT. Effect of anions on binding of bile salts by cholestyramine. Pharm Res 1991; 8:238-41.

4. Luner PE, Amidon GL. Equilibrium and Kinetic Factors influencing bile sequestrant efficacy. Pharm Res 1992; 9:670-6.

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